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Liquid / liquid interface-based electrochemical sensing of ractopamine and salbutamol

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Abstract

An electrochemical sensor based on ion transfer across micro- and nano-liquid / liquid interfaces has been developed for the detection of protonated drugs, ractopamine ($RacH^+$) and salbutamol ($SalH^+$) via cyclic voltammetry (CV) and linear sweep stripping voltammetry (LSSV). These voltammetric methods enabled the detection and characterisation of the ionised drugs despite that they transferred at high applied potentials. The drugs' thermodynamic and analytical parameters were determined. The limit of detection in the sub-μM range is suitable for applications to detection in real samples. Electrochemistry at the liquid / liquid interfaces was shown to be a viable technique for drug sensing.

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Keywords: electrochemical sensor; ion transfer; micro- and nano-liquid / liquid interfaces; protonated drugs; voltammetric methods

1. Introduction

Electrochemical sensing based on ion transfer across the interface between two immiscible electrolyte solutions (ITIES), or at liquid / liquid interfaces, has progressed from the transfer of model ions (e.g. tetraalkylammonium ions) to the detection of biologically relevant molecules (e.g. proteins, peptides, amino acids and other small molecules (drugs, neurotransmitters and food additives)) [1].

Ractopamine (Rac) and salbutamol (Sal) (or also known as albuterol) are β-adrenergic agonists with similar chemical structures and functions. These drugs were originally developed as therapeutic drugs in

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human and veterinary medicine for the treatment of pulmonary/respiratory diseases such as asthma. Unfortunately, β-adrenergic agonists are also illegally applied in the livestock industry as growth promoters, where they divert fat deposition to the production of muscle tissue. β-adrenergic agonist-treated animals may pose adverse effects on human health, especially in the cardiovascular and central nervous systems. As a result, this veterinary drug residue issue has recently become a public food safety concern [2, 3]. Therefore, rapid, simple and sensitive analytical methods for the detection and quantification of β-adrenergic agonists are vital.

A number of methods have been reported for the determination of ractopamine and salbutamol including immunoassays, electrochemical methods, spectrophotometry, high performance liquid chromatography, and so forth [3]. Even though spectrophotometry and chromatography are widely employed, these techniques are expensive and time-consuming (for derivatization steps) [4]. Electrochemical methods arise as advantageous due to low instrumental cost, fast analysis and simplicity. However, strong electrode fouling/poisoning reported for salbutamol detection is a drawback for solid / liquid interface based electrochemical sensing [4]. In addition, the ability of liquid / liquid interface-based electrochemical sensing to be implemented for detection of ionised ractopamine and salbutamol remains unexplored.

Herein, the electrochemical behaviour of $RacH^+$ at the micro- and nano-ITIES arrays, and $SalH^+$ at the micro-ITIES array via CV and LSSV is presented. Emphasis is placed on the electrochemical behaviour of the protonated drugs at the micro-ITIES array due to the issue of insufficient potential window for the transfer process. However, protonated drug detection at the micro-ITIES array will provide a basis for future studies at nano-ITIES array. The oxidation behaviour of ractopamine at the solid / liquid interface was also examined. In addition, the thermodynamic parameters for the transfer of ionisable ractopamine, and the influence of the interfering substances, including serum protein, towards $RacH^+$ detection were also studied.

2. Material and Methods

2.1. Materials and reagents

Chemical reagents used were obtained from Sigma-Aldrich Pty. Ltd., Australia. 10 mM lithium chloride (LiCl) prepared in ultrapure water, and 10 mM bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate (BTPPATPBCl) dissolved in 1,6-dichlorohexane (DCH), served as the aqueous and organic phase solutions, respectively. In the micro-ITIES study, the organic phase was present as an organogel with incorporation of low molecular weight poly(vinylchloride) (PVC) (10 % w/v). The organic reference solution consisted of 10 mM BTPPACl dissolved in 10 mM LiCl. Ractopamine hydrochloride and salbutamol, and tetrapropylammonium chloride (TPrACl) served as the drugs and model analyte species studied, respectively. The stock solutions of ractopamine hydrochloride and salbutamol were prepared in methanol (MeOH) due to their low solubility in water. In the study of the oxidation of ractopamine, the supporting electrolyte solution of 0.1 M phosphate buffered saline (PBS) (pH 7.4) was prepared in ultrapure water. 5 mM of interferents, prepared in 1 mM PBS solution were used in the interfering substances study. In the effect of artificial serum matrix towards $RacH^+$ detection, artificial serum compositions are as detailed previously [5].

2.2. Preparation of micro- and nano-interface arrays

The silicon micropore arrays used for micro-ITIES patterning were 11.09 ± 0.12 μm radius, r_a, 30 pores in a hexagonal array, and with pore centre-to-centre separation, r_c, of 18.4 ± 2.1 times the pore

radius, r_a (i.e. $r_c = 18.4r_a$) [5]. The nanopore arrays used for nano-ITIES patterning were fabricated in 50 nm thick silicon nitride membrane by focused ion beam (FIB) milling methods as have been reported recently [6]. The nanopores were 39 ± 6 nm in r_a, 400 pores in cubic array, and with r_c of 20 ± 3 times r_a.

2.3. Experimental procedure at the micro- and nano-ITIES arrays

The procedure applied when preparing the set-up for micro-ITIES was similar to nano-ITIES, with the major difference being the membrane used to support the miniaturised ITIES, as outlined previously [5]. The main cell set up can be schematically summarised as follows:
Ag / AgCl / x M drug + 10 mM $LiCl_W$ // 10 mM $BTPPATPBCl_{DCH}$ / 10 mM BTPPACl in 10 mM $LiCl_W$ / AgCl / Ag
where x is the concentration of drug in the aqueous phase (ractopamine or salbutamol). As a control of the potential axis, $TPrA^+$ was spiked into the aqueous phase, normally after the final drug or interferent injection.

Voltammetry experiments (CV and LSSV) at the micro- and nano-ITIES array were performed using an Autolab PGSTAT 302N (Metrohm Autolab B. V., Utrecht, The Netherlands) interfaced to a personal computer with Nova 1.8 software for data processing. A background voltammogram was recorded over a wide potential range to establish the available potential window limits before injection of the required amount of analyte concentrated solution into the aqueous phase. Throughout this study, a voltammetric sweep rate of 5 mV s^{-1} was employed.

2.4. Experimental procedure at the solid electrode

A conventional three electrode system was employed. The working and reference electrodes were glassy carbon electrode (GCE) and commercial Ag/AgCl electrode, respectively (CH Instruments Inc., Austin, USA). The counter electrode was a platinum wire. CV of 20 to 100 µM ractopamine was recorded from 0.2 to 0.9 V, and the sweep rate, v was 50 mV s^{-1}.

3. Result and Discussion

3.1. CV of $RacH^+$ transfer at the micro- and nano-ITIES arrays, and $SalH^+$ transfer at the micro-ITIES array

The CVs of $RacH^+$ and $SalH^+$ transfers at the micro-ITIES array (Fig 1(a)) show that these ions, which are initially present in the aqueous phase, are transferred into the organic phase, on the forward CV scan. On the reverse scan, these ions are transferred back from the organic into the aqueous phase. However, the ion transfer waves on forward CV scan were close to the positive limit of potential window, with foot potentials of 0.8 V and 0.85 V for $RacH^+$ and $SalH^+$ ions, respectively. The current increased steadily with applied potential up to the switching potential. On the reverse CV scan, peak-shaped features were observed, due to the slower ion diffusion rate in the organogel [5]. This study focused on $RacH^+$ detection due to the lower current magnitude detected for $SalH^+$.

The CV of $RacH^+$ transfer at the nano-ITIES array (Fig 1(b)) was investigated to see if the enhanced diffusional mass transport flux improved the detection performance. The forward CV scan showed a similar behavior as observed at the micro-ITIES array, with a similar ion transfer wave foot potential (0.8 V). Conversely, on the reverse scan, no peak behavior was observed as at the micro-ITIES array. Comparison of the current density signal generated at the micro- and nano-ITIES arrays showed a 5-times higher signal for the latter than the former, respectively. The current density improvement with this nano-

ITIES array serves as a basis for sensitivity and detection limit measurement, which remains part of future investigations.

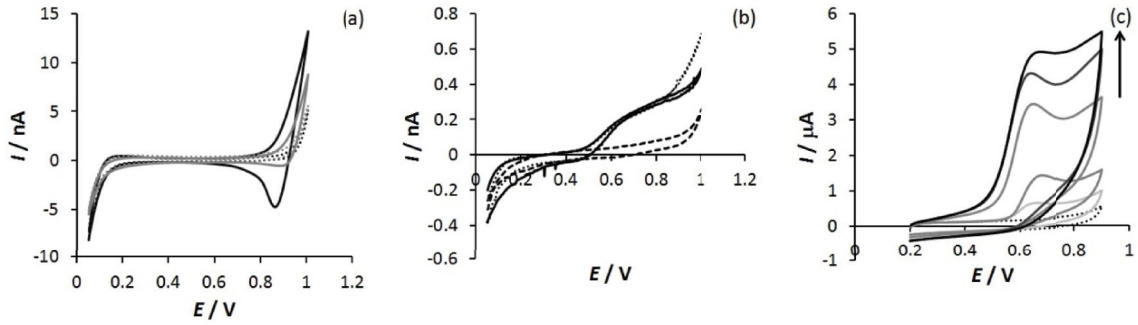

Fig. 1. (a) CVs of 100 µM $RacH^+$ (black, solid line) and $SalH^+$ (gray, solid line) transfer at the micro-ITIES arrays. CVs of $RacH^+$ (black, dotted line) and $SalH^+$ (gray, dotted line) blank; (b) CVs of 100 µM $RacH^+$ transfer spiked after $TPrA^+$ injection at the nano-ITIES array. Dashed, solid and dotted lines represent the blank aqueous phase solution, 100 µM $TPrA^+$, and 100 µM $TPrA^+$ + 100 µM $RacH^+$, respectively; (c) CVs of 20 (light gray) to 100 µM (black) $RacH^+$ oxidation in step of 20 µM. Dotted line represents blank voltammogram, arrow indicates increasing analyte concentrations

3.2. Oxidation response of $RacH^+$

In the study of the oxidation of $RacH^+$, an oxidation peak was observed at 0.65 V, however no reduction peak was observed on the reverse scan, suggesting an irreversible oxidation of $RacH^+$ (Fig. 1(c)). The $RacH^+$ oxidation peak current decreased significantly during the second anodic scan, indicating fouling of the electrode surface, perhaps due to strong adsorption of the $RacH^+$ oxidation product.

3.3. Thermodynamic parameters of $RacH^+$ transfer

Besides analytical studies, the ability of the ITIES to mimic drug transfer across biological membranes can offer insight into mechanisms of drug action. A number of thermodynamic parameters for $RacH^+$ transfer can be determined from the CV data. The values of the formal transfer potential ($\Delta_0^w \emptyset^{o\prime}$), the formal Gibbs energy of transfer ($\Delta G_{transfer}^{0\prime,w\rightarrow DCH}$) and the partition coefficient ($\log P_{DCH}^0\ (ionised)$) of $RacH^+$ are 0.29 V (in DCH), 27.88 kJ mol^{-1} and -4.89, respectively, as detailed previously [5].

3.4. LSSV of $RacH^+$ transfer at the micro-ITIES array

LSSV was employed in order to detect lower concentrations relative to CV, as this entails a pre-concentration step that enhances sensitivity. The optimized parameters (5 mV s^{-1} scan rate, 1.00 V pre-concentration potential, 120 s pre-concentration time) were used, and a calibration graph of stripping peak current versus concentration was plotted. The calculated limit of detection (LOD) is 0.1 µM (34 ng mL^{-1}), based on 3 times the standard deviation of the blank ($3s_b$) which compares well with published values of 0.05 – 1.17 µM at various solid electrodes [5].

3.5. Impact of the potentially interfering substances on $RacH^+$ detection

Possible interferences in the detection of $RacH^+$ were investigated using models of substances likely to be present in a biological samples such as sugar (glucose), ascorbic acid, metal ions (K^+ and Na^+), an amino acid (glycine) and urea. None of the substances was seen to significantly decrease the available potential window nor to introduce new peaks in the voltammograms [5]. To investigate the possibility that protein can impede the detection of $RacH^+$, artificial serum solutions, with and without bovine serum albumin (BSA, a water-soluble protein) at physiological concentration were prepared. Potential window shortening, binding of the $RacH^+$ by BSA, and adsorption of BSA to the interface are the possible interference mechanisms for the detection of $RacH^+$ in serum via ion-transfer voltammetry. Hence, sample deproteinisation is necessary for the viability of this voltammetric analysis [5].

4. Conclusion

The electrochemical detection of protonated ractopamine drug ($RacH^+$) was achieved by means of CV and LSSV at the micro-ITIES array. $RacH^+$ transferred close to the positive limit of the potential window. In addition, $RacH^+$ and $SalH^+$ transfer at the nano- and micro-ITIES arrays, respectively, were feasible. As a comparison, an irreversible oxidation of ractopamine was observed at the solid / liquid interface. The determination of $RacH^+$'s thermodynamic parameters was possible via CV. LSSV with optimised conditions enabled a limit of detection of 0.1 μM to be achieved, which is suitable for drug detection in real samples. The potential window was not interfered significantly by substances such as a sugar, ascorbic acid, metals, an amino acid and urea. Serum protein present in an artificial serum solution impeded the $RacH^+$ detection signal via potential window reduction, drug-protein complexation and adsorption at the ITIES, hence a deproteinisation step is suggested before bio-sample analyses.

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